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INFLAMMATION AND PLAQUE VULNERABILITY

Göran K Hansson[1], Peter Libby[2] and Ira Tabas[3]

1) Department of Medicine and Center for Molecular Medicine, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden;
2) Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA;
3) Department of Medicine, Department of Pathology and Cell Biology, and Department of Physiology, Columbia University Medical Center, New York, NY, USA.

Correspondence:

Professor Göran K Hansson
Center for Molecular Medicine L8:03
Karolinska University Hospital
SE-17176 Stockholm
Sweden
e-mail: goran.hansson@ki.se

Atherosclerosis: chronic inflammation in the artery wall

Atherosclerosis is a maladaptive, non-resolving chronic inflammatory disease that occurs at sites of blood flow disturbance. The atherogenic process is thought to be triggered by the subendothelial retention of cholesterol-containing plasma lipoproteins at these sites and by flow-mediated inflammatory changes in endothelial cells [1, 2] . The lesions contain monocyte-derived macrophages and T cells interspersed with acellular regions containing lipids and debris from dead cells, embedded in an extracellular matrix composed of collagen fibers and other constituents produced primarily by vascular smooth muscle cells[3, 4]. The collagenous matrix typically forms a fibrous cap that overlies the lipid rich region in the plaque core. Lesions generally remain covered by an intact endothelium until the late stages of the disease. The eventual breakdown of endothelial continuity can promote lesion progression and complication.

Cells of the atherosclerotic lesion display features of ongoing inflammation, with macrophages and T cells producing a host of mediators including proinflammatory cytokines, costimulatory factors for immune activation, eicosanoids, and reactive oxygen and nitrogen species [5, 6]. In addition, many of the macrophages internalize cholesterol through their scavenger receptors and some also produce anti-inflammatory cytokines. Furthermore, certain T cells of the regulatory phenotype display anti-inflammatory and immunosuppressive features. This delicate balance between pro- and anti-inflammatory signals results in a slowly progressive, non-resolving, chronic inflammation[7].

Innate and adaptive reactions in the artery

Several reactions link lipid accumulation to inflammation. In the macrophage, pattern recognition receptors selected in evolution for handling components of microbial pathogens also mediate internalization of modified lipoproteins[5, 6]. These scavenger receptors evade suppression due to increases in intracellular cholesterol concentrations, and can therefore mediate continued lipoprotein uptake that permits overloading the cell with lipids. At a certain point, intracellular cholesterol precipitates as microcrystals. Analogously with urate

crystals, these cholesterol microcrystals can activate an inflammasome, i.e. a cytosolic molecular machine that cleaves a proform of interleukin (IL)-1 beta, converting it into bioactive IL-1beta that can be secreted by the cell[8]. When released in the arterial intima, IL-1beta induces production of a set of other proinflammatory molecules, including the cytokine IL-6 and the proinflammatory eicosanoid, PGE_2[9, 10]. IL-1 beta also promotes expression of leukocyte adhesion molecules and matrix-degrading metalloproteinases. Thus, cholesterol accumulation begets inflammation and tissue remodelling.

Another set of pattern recognition receptors, the Toll-like receptors, may bind modified lipoprotein particles in the arterial intima[11-14], triggering phosphorylation cascades that elicit expression of a set of proinflammatory genes similar but not identical to that elicited by IL-1 beta. For instance, TNF induces expression of matrix metalloproteinases that degrade collagen and promotes tissue remodelling[15]. TNF has crucial pathogenetic importance in rheumatoid arthritis and other inflammatory diseases and also impacts atherosclerosis substantially [16-18].

Presentation by dendritic cells of fragments of LDL particles to T cells in lymph nodes draining the atherosclerotic lesion calls adaptive immunity into action [19, 20]. Clones of T cells that recognize peptide fragments of the main LDL apoprotein (apoprotein B) that can act as autoantigens. This encounter tends to differentiate the T cells into proinflammatory Th1 effector cells under the influence of proinflammatory mediators such as IL-12 found in plaque[20, 21]. Effector T cells patrol the body, enter at sites such as the plaque, where endothelial cells express leukocyte adhesion molecules. These T cells may undergo reactivation by LDL fragments. Such renewed activation prompts the Th1 cell to produce large amounts of TNF and also another proinflammatory cytokine, interferon-gamma [21, 22]. This interferon strongly stimulates macrophages and also profoundly effects vascular endothelial and smooth muscle cells, causing them to express leukocyte adhesion molecules, modulate their fibrinolytic properties, reduce proliferation, and in the case of the smooth muscle cell, inhibit fibrillar collagen formation[23, 24]. Interestingly, in keeping with the counterbalancing forces mentioned above, lesional dendritic cells can

also promote the development of pro-resolving regulatory T cells in early atherosclerosis[25, 26] , but ultimately the effector:regulatory T cell balance promotes progressive inflammation.

In the advanced atherosclerotic plaque, infiltrating mast cells contribute to the proinflammatory milieu[27]. Upon activation, these cells release a host of mediators and enzymes, including histamine, serotonin, thromboxane and other eicosanoids, cytokines, and a set of serine proteases, all of which may profoundly affect the atherosclerotic lesion.

The concerted action of all proinflammatory signals operating in the plaque not only enhances inflammation but also hampers renewal of the structural elements that support the mechanical stability of the inflamed tissue.

Clinical and histopathological features of culprit lesions

The atherosclerotic process typically lies silent for months, years, and even decades, and may never result in clinical manifestations[2]. Yet, if the plaque's surface is damaged, thrombotic occlusion of the artery may ensue. Surface continuity may be damaged by fissuring (so-called plaque rupture, observed in 60 to 80% of cases of acute coronary syndrome) or surface erosion (present in 20 to 40% of cases with coronary thrombosis, especially in women and young victims of sudden coronary death)[28, 29]. Figures 1-2 depict these two different types of discontinuity of the plaque surface. Recent studies suggest that the proportion of infarctions caused by rupture vs erosion is changing, with more cases due to erosion and fewer to overt plaque rupture[30].

Fissures and erosions trigger atherothrombosis by exposing thrombogenic material inside the plaque, such as phospholipids, tissue factor, and matrix molecules, to platelets and coagulation factors[2]. Platelet aggregates precipitating on these exposed surfaces are stabilized by fibrin networks. Tissue factor, expressed by macrophages and by vascular smooth muscle cells in the atherosclerotic plaque, can initiate the blood coagulation cascade that leads to fibrin formation[31]. Atherothrombi expand rapidly and can fill the lumen within minutes, thereby leading to ischemia and infarction.

A range of factors may contribute to atherothrombosis. Disturbance of the balance between prothrombotic and fibrinolytic activity on the plaque surface probably plays an important role for precipitating the thrombotic event[32], but the precise sequence of events that operate in vivo is not yet known.

The "vulnerable plaque"

Thrombi precipitate on damaged vascular surfaces, as recognized by Rudolf Virchow in 1856[33]. The cause of the damage leading to plaque rupture or erosion remains incompletely understood, despite considerable progress in this regard. Constantinides, Davies, Falk and their colleagues observed that ruptured plaques display thin fibrous caps and large lipid core regions[34-36]. These findings highlighted structural abnormalities in the vessel wall as a cause of atherothrombosis. Subsequent investigations have revealed that culprit lesions of fatal thrombi in coronary arteries contain reduced amounts of mature, crosslinked collagen and increased levels of collagen-degrading enzymes.

In vivo imaging technology now offers approaches to the analysis of major plaque components. For example, optical coherence tomography (OCT) and magnetic resonance imaging can identify thin-cap plaques. Computerized tomographic angiography can identify outward arterial remodelling, radiolucency, and spotty calcification associated with coronary events. Such approaches, albeit incompletely validated, currently see used to obtain surrogate endpoint data on effects of putative plaque-stabilizing therapies[37-39].

Histopathologic analysis of lesions that have provoked fatal myocardial infarction (MI) shows stimata of inflammation including accumulation of macrophages, activated T cells, dendritic cells, and mast cells as well as reduced thickness of the fibrous cap and increased neovascularization at sites of plaque rupture and thrombosis[40] (Fig. 1). Matrix metalloproteinases and cysteine proteinases, products of activated macrophages, localize at sites of plaque rupture[41]. Several of these enzymes digest fibrillar collagen, thus reducing the mechanical stability of the plaque[41, 42]. These proteinases likely render plaques susceptile to rupture, but have complex effects on the composition and size of lesions in mouse experiments.

Lesional cell death

Cell death may also predispose to plaque rupture[7, 43]. Smooth muscle cells (SMC) synthesize the bulk of the arterial extracellular matrix. Site of fatal plaque rupture display depletion of SMC needed to repair and maintain the collagen that comprises the plaque's fibrous cap. Apoptosis of SMC documented in athromata, may thus lead to their relative lack at sites of plaque rupture. Rapid phagocytosis usually clears the remnants of cells that have undergone apoptosis, a process know as efferocytosis[44]. If this process fails, secondary necrosis ensues, contributing to the formation of the plaques lipid core, also known as the "necrotic core." Computational analyses indicate that lipid core accumulation can reduced the mechanical integrity of the plaque.

Plaque necrosis results from death of lesional cells, mostly macrophages. Cell death can lead to necrosis by at least two mechanisms: apoptosis followed by defective phagocytic clearance ("efferocytosis") of the apoptotic cells; and a process called primary necrosis [7] . Macrophage apoptosis occurs in lesions of all stages. A number of plaque factors are likely to trigger lesional macrophage apoptosis, including excessive inflammation, oxidized lipids, and cholesterol, often in combination through a "multi-hit" process. Observational data in human atheromata and molecular-genetic causation data in mouse models of advanced atherosclerosis indicate that one of the hits casued by these factors in chronic endoplasmic reticulum (ER) stress [45] . In particular, the ER stress effector CHOP is tightly associated with cell death and plaque necrosis in human coronary artery lesions, and genetic deletion of CHOP in mice protects against advanced lesional macrophage apoptosis and plaque necrosis [45] .

In early atherosclerosis, the apoptotic cells are properly cleared by neighboring phagocytes, which prevents post-apoptotic necrosis and triggers pro-resolving processes that are linked to efferocytosis [46].In advanced plaques, however, efferocytosis is defective, leading to cell necrosis, release of pro-inflammatory damage-associated molecular patterns (DAMPs), and lack of efferocytosis-mediated pro-resolving signaling [47-49]. Collectively, these processes promote the type of inflammatory, necrotic lesions that are charcateristic of vulnerable plaques (see below). The mechanisms of defective efferocytosis in advanced

atherosclerotic lesions are not known and are likely to be multi-factorial. A recent study provided correlative evidence in human atheromata suggesting a role for ADAM17-mediated cleavage of MerTK, a macrophage efferocytosis receptor shown to be important in the progression of murine atherosclerosis [49-51]. It is also interesting to note that defective efferocytosis is a cardinal sign of defective inflammation resolution [52], and that a therapeutic strategy that enhanced resolution in advanced murine plaques markedly suppessed plaque necrosis [53] .

Whereas, defective efferocyosis leads to plaque necrosis through secondary necrosis of uncleared apoptotic cells, cells can undergo another process in which necrosis develops as a primary event. In this case, a signaling cascade involving RIP1 and RIP3 kinases is involved, and when RIP3 kinase was genetically targeted in fat fed LDL receptor null mice, plaque necrosis was partially suppressed[54]. These data suggest that, at least in advanced murine atheroma, both secondary and primary apoptosis contribute to plaque necrosis.

Plaque erosion

Plaques that have disrupted due to fibrous cap fracture tend to have a large lipid core[30], and the potent procoagulant tissue factor localizes in these cores[7] (Fig. 1). Those disrupted by erosion, another substrate for thrombus formation, do not have a large lipid core and show less inflammatory cell accumulation than fissured plaques (Fig. 2). Plaques frequently rupture without clinical manifestations, possibly reflecting variation in the thrombotic response depending on the thrombogenicity of exposed plaque constituents, local hemorheology, shear-induced platelet activation systemic clotting activity, fibrinolytic function, and sensitivity of the end organ to ischemia.

Plaques displaying endothelial erosion seem to differ from rupturing ones in some important aspects[29]. They appear to be less inflamed but contain proliferating smooth muscle cells, abundant proteoglycans and hyaluronan, and substantial neovascularization. Therefore, pathogenetic mechanisms may differ between these two conditions and we will consider them separately.

Why do plaques rupture?

Most of our knowledge about plaque rupture comes from studies of human autopsy specimens and surgical material. Key histopathological findings associated with regions of fatal disruption include a thin fibrous cap (< 50-60 microns), increased signs of inflammatory activity, and heightened amounts of proteolytic enzymes [55-59]. Therefore, inflammatory stimuli such as local immune reactions might activate macrophages, mast cells and T cells to release cytokines that inhibit cap formation and proteases that digest fibrous components of the cap (Fig. 1).

Much interest has focused on the collagenolytic action of matrix metalloproteinases and cysteine proteases in the plaque. A set of such enzymes are present in the human atherosclerotic plaque and has shown proteolytic activity in culprit lesions[57, 60]. These findings have encouraged attempts at developing plaque-stabilizing therapies by targeting proteases. Several excellent reviews cover this interesting development in detail[61, 62].

A set of immune cytokines impacts powerfully on the fibrous cap (Fig. 1). Interferon-gamma, a proinflammatory, macrophage-activating cytokine produced by Th1-type T cells and NK cells, inhibits collagen fiber formation, causing plaques to adopt a vulnerable phenotype with reduced collagen content. This is due to a triple action of interferon-gamma, as it both inhibits smooth muscle differentiation[24], procollagen-I gene expression[23] and the collagen cross-linking enzyme, lysyl oxidase[63].

The action of Th1 cells is counterbalanced by Treg cells producing TGF-beta[64] (Fig. 1). This cytokine has a direct, fibrogenic action on smooth muscle cells and fibroblasts. In addition, it inhibits Th1 and macrophage activity, leading to reduced plaque inflammation. Treg also enhance the catabolism of very-low density lipoproteins, resulting in reduced plasma lipid levels.

A third type of T cells, the Th17 cell type, is involved in wound healing and exerts powerful fibrogenic activity[65]. Th17 cells activated in the context of atherosclerosis promote the formation of thick collagen fibers that can withstand the mechanical assault on the plaque exerted by hemodynamic forces[66]. This is

due to the capacity of the signature Th17 cytokine, IL-17A, to promote procollagen expression (Fig. 1).

In addition to reducing the capacity of the tissue to withstand mechanical strain, immune signals may also promote atherothrombosis by increasing the tendency to form platelet aggregates and clots (Fig. 1). The TNF/TNF receptor superfamily members, CD40 ligand (CD40L, CD154) and CD40, may have particular importance in this context. CD40L, typically expressed on activated T cells, ligates CD40 on cells of the macrophage lineage. This stimulation triggers expression of tissue factor as well as matrix metalloproteinase secretion[67]. In addition, activated platelets also express CD40L [68] and endothelial cells exhibit its receptor CD40 [69], allowing for multiple heterophilic interactions that may promote atherothrombosis[70, 71].

Lipid mediators are at least as important as cytokines in the sequence of events leading to atherothrombosis (Fig. 1). The prothrombotic effect of thromboxane A2 released from platelets and the counterbalancing, antithrombotic effect of endothelium-derived prostaglandin I2 (PGI2, a.k.a. prostacyclin) is well known, crucial for vascular homeostasis, and the target of aspirin used in cardiovascular prevention[72, 73]. Other prostaglandins play different roles in the atherosclerotic artery wall. Thus, PGE2 produced by several cell types promotes vasodilation and macrophage activation but also increases expression of the antiinflammatory cytokine, IL-10[74].

The leukotriene pathway of lipid mediators also exerts powerful effects on atherosclerosis. Leukotriene B4 is a proinflammatory leukotriene expressed in plaques[75, 76]. Through its BLT1 receptor, it promotes plaque growth and enhances its inflammatory properties[77]. It also increases vascular restenosis after endothelial injury[75]. 5-lipoxygenase-activating protein (FLAP), a co-factor for the enzyme that converts arachidonic acid into the leukotriene pathway, is upregulated in plaques and promotes leukotriene(LT)B4 production[78]. Genetic polymorphism in the FLAP encoding gene, ALOX5AP, was associated with cardiovascular disease in several genetic studies[79], although it did not turn out to be a major genetic risk factor in genome-wide association studies. However, this does not rule out a possible role for leukotriene signaling in cardiovascular

disease. As many patients with asthma are treated with leukotriene receptor blockers, long-term follow-up of these individuals permits an assessment of the importance of leukotriene signaling in cardiovascular disease[80]. A population-based Swedish study of 7 million cases revealed that those treated with the leukotriene receptor blocker, montelukast had a 35% reduced risk of recurrent stroke and myocardial infarction[80].

Lipoxins and resolvins produced in the 12/15-lipoxygenase pathway counterbalance the proinflammatory effects of leukotrienes and may inhibit atherosclerosis and its clinical complications [81]. In line with this notion, targeting the lipoxin receptor FPR2/ALX by genetic abrogation leads to features of reduced plaque stability [82]. Further studies will be required to clarify the role of pro- and anti-inflammatory lipoxygenase products in atherosclerosis.

Clinical studies have associated ischemic atherothrombotic events such as MI and stroke with infections. Acute infections, via elicitation of systemic cytokines, may elicit an "echo" of inflammatory activation in the plaque, leading to bursts of proinflammatory, proteolytic, and prothrombotic activity, although we currently lack definitive evidence to confirm such a chain of events[83].

The lack of suitable animal models has hampered research on plaque disruption. Although under circumstances that should promote thrombosis on plaques in rodents, such experiments yielded a low incidence of thrombosis and lack of linear relationship between events and histopathological findings such as "buried caps"[84-86]. Such studies have not generally dealt with coronary arteries, rather the aorta or its large caliber branches. Yet, more recent work has described promising experimental preparations that may be more suitable for addressing mechanisms of plaque rupture[87]. In genetically hypercholesterolemic mutant mice, several interventions can precipitate rupture of existing atherosclerotic plaques, for example virally-directed local overexpression of an active form of the MMP stromelysin, the long-term infusion of angiotensin II[88], placement of a cuff around the carotid artery[89], partial ligation of this artery[90] or increasing elastin fragmentation through a "knock-in" mutation in the fibrillin-1 gene[91]. Yet, none of these preparations induces standardized plaque ruptures at

a given time in a controlled manner. Instead, they increase the tendency for the plaque to rupture, and heal, spontaneously.

Signs of plaque rupture include intraplaque haemorrhage, fractured cap fibers, and multi-layered "buried" caps[92]. Enumeration of these signs by microscopy permits quantification of the phenomenon. Such methods have obvious limitations but may permit investigators to assess the effects of various treatments on the tendency for plaques to rupture. The contrived nature of these manipulations, however, limits the generalizability of such experiments. For example, a blocker of angiotensin II should limit disruptions produced by infusions of this mediator, and MMP inhibitors will reduce the consequences of stromelysin overexpression with no predictive value for the effects of these interventions on plaque rupture in humans.

Why does the endothelium erode?

Mechanisms instigating endothelial erosion have been unclear. However, recent studies point to a role for innate immunity in this process (Fig. 2). Endothelial cells overlying atherosclerotic lesions abundantly express the pattern recognition receptor, Toll-like receptor-2 (TLR2)[11]. Ligation of this receptor results in endothelial apoptosis in a process accelerated by polymorphonuclear leukocytes, a cell type found at sites of fatal plaque erosion[93]. TLR2 ligands include the extracellular molecule hyaluronan as well as components of Gram-positive bacteria[94], therefore endogenous as well as infectious factors may operate to promote atherothrombosis through this mechanism[93]. Stressful events also associate with acute ischemic events. For example, the incidence of myocardial infarction often rises shortly after major sports events (particularly in males), and peaked after stressful events such as a major earthquake[95, 96]. This association may result from acute changes in local hemodynamics of the atherosclerotic artery. Exposure of atherosclerotic mice to stressful stimuli led to endothelin-dependent vasoconstriction that preceded thrombosis and myocardial ischemia, possibly because the vasoconstrictive episode had caused endothelial erosion[56] . Likewise, infusion of spasmogenic stimuli in MI-prone rabbits elicited occasional coronary artery thrombi resembling human superficial erosion[97].

How can plaques be stabilized?

Abundant experimental and some clinical data using MRI or intravascular imaging suggest that lipid lowering, and statin therapy in particular may alter plaque properties implicated in susceptibility to rupture. Several other approaches may stabilize plaques (Fig. 3). None of them have entered clinical trials on the indication to stabilize plaques, in part due to the difficulties in identifying vulnerable, ruptured, eroded and thrombosed lesions in the living patient. Current progress in in vivo imaging techniques might enable such trials in the future.

Conclusion

Atherosclerosis associates strongly with systemic risk factors (e.g. high LDL, hypertension, diabetes), yet the lesions distribute multifocalally. Most plaques remain silent throughout life but certain individual lesions may provoke thrombotic complication and ischemia, resulting in life-threatening complications. The discovery of plaque rupture and endothelial erosion as two main causes of atherothrombosis helps us to understand why this very chronic condition manifest clinically in an episodic and unpredictable fashion. Further studies have clarified that inflammation, proteolysis, and reduced collagen fiber content predispose to plaque rupture, whereas endothelial erosion followed by neutrophil infiltration typically complicates lesions of a distinct morphology. Lack of animal preparations that develop disruption of atherosclerotic plaques has, however, hampered progress in mechanistic research on atherothrombosis. Similarly, limitations of non-invasive in vivo imaging of so called “vulnerable plaques” in humans has hampered clinical work in this domain. Recent progress in both these areas may address these issues and aid the development and evaluation of plaque-stabilizing therapies beyond lipid lowering in the forthcoming years. The many unanswered questions in this field provide ample opportunity for future research, and may yield avenues to improve patient outcomes.

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Figure legends:

Figure 1. Mechanisms of plaque rupture.

Activated macrophages and Th1 cells produce metalloproteinases and cytokines that hamper the tensile strength of the collagen cap. Several proinflammatory cytokines including interferon-g (IFNg) and tumor necrosis factor (TNF), as well as CD40/CD40L cell surface receptors of the TNF superfamily promote an inflammatory state that enhance cell death and prothrombotic activity in the plaque. When the cap no longer can withstand the mechanical force of the blood pressure, superficial fissures are formed in the plaque. Exposure of the plaque's inner core with its thrombogenic material rapidly triggers platelet activation, humoral coagulation, and the formation of a thrombus that may either occlude the artery at the site of plaque rupture, or dissociate as an embolus and occlude the arterial lumen at a site downstream of the ruptured plaque.

Counteracting all these proinflammatory and tissue-destructive signals, subsets of macrophages and T cells produce anti-inflammatory molecules that counteract vascular inflammation and reduce the risk for plaque rupture and atherothrombosis. Among them, transforming growth factor-b (TGF-b) and interleukin-10 (IL-10) inhibit inflammation and immune cell activation. In addition, TGF-b has fibrogenic properties that it shares with IL-17A produced by Th17 cells. The resolution of plaque inflammation depends not only on anti-inflammatory signals but also on resolving mediators such as eicosanoids of the resolvin type and Annexin I, both of which ligate the FPR/ALX receptor.

EC, endothelial cell; SMC, smooth muscle cell; MΦ, macrophage; MMP, metalloproteinase; TXA_2, thromboxane A_2; PGI_2, prostaglandin I_2 (prostacyclin).

Figure 2. Mechanisms of plaque erosion.

Endothelial cells of atherosclerotic plaques commonly express Toll-like receptor –2 (TLR2) that can ligate both Gram-positive toxins (G+ toxins) of bacterial pathogens and hyaluronan released from the extracellular matrix. TLR2 ligation can trigger endothelial dysfunction with endoplasmic reticulum stress and apoptosis. Such reactions are further enhanced by neutrophil attack on the endothelium. As a result, endothelial cells may detach, exposing the

subendothelial matrix with its thrombogenic components. Activated neutrophils contribute to a prothrombotic state by releasing a set of proteases including neutrophil elastase and by forming neutrophil extracellular traps (NETs) that can damage endothelial cells, trap leukocytes, and enhance thrombosis.
PAD4, Peptide arginine deaminase-4, a component of NETs.

Figure 3. Therapy targets for prevention of atherothrombosis.
Reduction of LDL (and other large lipoproteins) by lipid-lowering therapy, and prevention of LDL retention in the artery wall, both act to reduce cholesterol accumulation, an initiator of atherosclerosis. Stimulation of immunoregulatory mechanisms reduce vascular inflammation; they include administration of anti-inflammatory cytokines, enhancing Treg cells, and vaccination to elicit atheroprotective immunity. Mediators of resolution include resolvin-type eicosanoids, peptide mimetics of Annexin I, and other substances.

Figure 1.

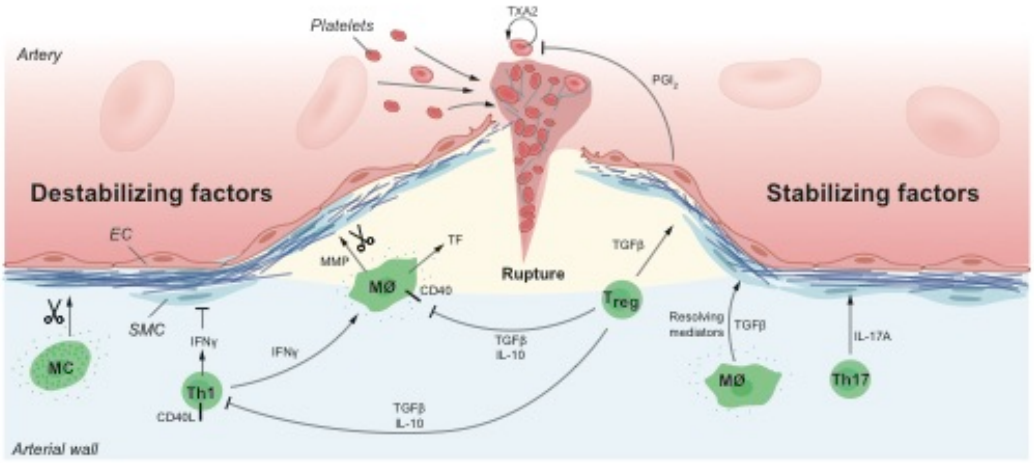


Figure 2.

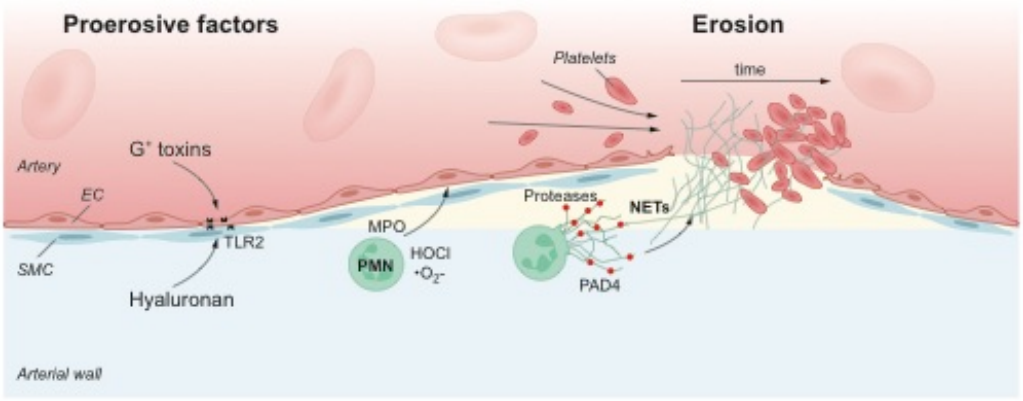
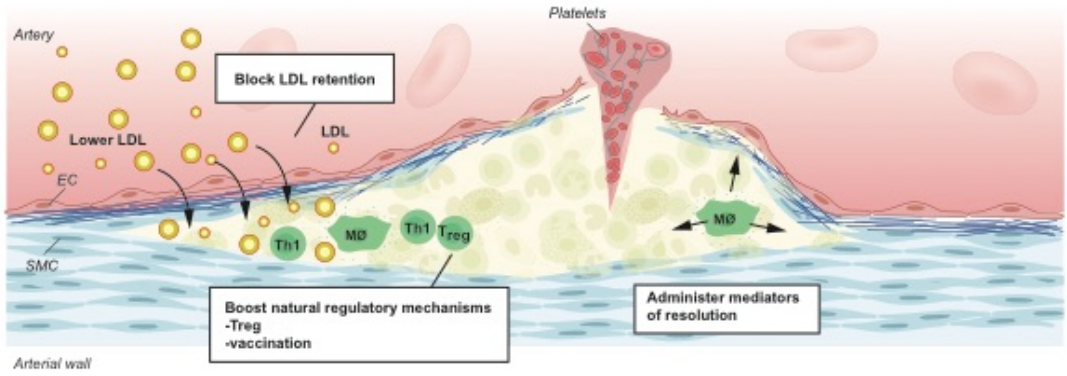


Figure 3.


Artery
Block LDL retention
LDL
Lower LDL
EC
SMC
Th1
MØ
Th1
Treg
Platelets
MØ
Boost natural regulatory mechanisms
-Treg
-vaccination
Administer mediators
of resolution
Arterial wall